Data Evaluation Report on the Acute Dietary Toxicity of Bitrex® (Denatonium Benzoate) to Mallard Duck (*Anas platyrhynchos*)

MRID 47146907

Data Requirement:

DP Barcode:	D340828
MRID:	47146907
Guideline:	850.2200

Test material: Bitrex® **Purity:** 100.2%
Common name: Denatonium benzoate
Chemical name: IUPAC: Benzyl diethyl ((2,6-xylylcarbamoyl)methyl) ammonium benzoate
CAS name: N-(2-((2,6-dimethylphenyl)amino)-2-oxoethyl)-N,N-diethylbenzene methanminium benzoate
CAS No.: 3734-33-6
Synonyms: Denatonium benzoate; lignocaine benzyl benzoate

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Staff Scientist, Dynamac Corporation

Signature: Christie E. Padova
Date: 8/31/07

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EPA PC Code: 009106

Date Evaluation Completed: 2/28/14

CITATION: Hakin, B., and A.J. Johnson. 1992. Bitrex® - Subacute Dietary Toxicity Study (LC_{50}) to Mallard Duck. Unpublished study performed by Huntingdon Research Centre Ltd., Huntingdon, United Kingdom. Laboratory Study No. MCS 7/920419. Study sponsored by Johnson Matthey Macfarian Smith, Edinburgh, United Kingdom. Study initiated January 20, 1992 and completed June 23, 1992.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute dietary toxicity of a pesticide to avian species. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY

The acute dietary toxicity of Bitrex® (denatonium benzoate) to 10-day old mallard duck (*Anas platyrhynchos*) was assessed over 8 days. Bitrex® was administered to the birds in the diet at nominal concentrations of 0 (negative control), 650, 1300, 2600, and 5200 mg ai/kg diet. Mean-measured concentrations were <12 (<LOD, control), 680, 1310, 2540, and 5150 mg ai/kg diet, respectively. No treatment-related effects were observed on mortality, body weight, or feed consumption. No clinical signs of toxicity were observed and no treatment-related observations were made at necropsy. The observed 8-day acute dietary LC_{50} >5150 mg ai/kg diet and the NOAEC = 5150 mg ai/kg diet (visual determination). According to the US EPA classification, Bitrex® is classified as practically non-toxic to mallard duck (*Anas platyrhynchos*) on an acute dietary basis.

This toxicity study is classified as **Acceptable** and satisfies the guideline requirement for an acute dietary toxicity study with mallard duck.

Results Synopsis

LC_{50} > 5150 mg ai/kg diet 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A

NOAEC = 5150 mg ai/kg diet
LOAEC > 5150 mg ai/kg diet

Endpoints Affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the U.S. EPA Pesticide Assessment Guidelines 71-2. Deviations from OCSPP Guideline 850.2200 included:

1. It is unknown if the study was conducted in compliance with GLP Standards
2. Four concentration levels were tested; a minimum of five concentration levels is recommended for the definitive study.
3. The photo-period was 12 hours light:12 hours dark; at least 14 hours light or continuous lighting is required.

These deviations do not affect the scientific soundness of the study.

COMPLIANCE: Signed and dated Quality Assurance and Data Confidentiality statements were provided. The submitter reported that it was unknown if the study was conducted in compliance with U.S. EPA GLP Standards (40 CFR Part 160).

A. MATERIALS

1. Test Material Bitrex® (denatonium benzoate)

Description: White granular solid

Lot No./Batch No. : 19667

Purity: 100.2%

Stability of compound under test conditions: The stability of Bitrex® was verified in the treated feed prepared at 163 and 5200 mg ai/kg diet for 6 days at ambient temperature. Mean recoveries were 99-103% of initial mean concentrations.

Storage conditions of test chemicals: Room temperature in the dark

Physicochemical properties of Bitrex® (Denatonium Benzoate)

Parameter	Values	Comments
Water solubility at 20°C	45 g/L	Temperature not specified (MRID 47135818)
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

2. Test organism

Species (common and scientific names): Mallard duck (*Anas platyrhynchos*)

Age at study initiation: 10 days old

Weight at study initiation (mean and range): group means of 122-132 g

Source: Mr. John Coles, The County Game Farms, Ashford, Kent, England.

B. STUDY DESIGN

1. Experimental Conditions

a. Range-finding Study

An initial range-finding study was conducted at nominal levels of 5 to 5200 mg ai/kg diet; no treatment-related effects were observed. The concentrations selected for use in the definitive study were based upon the results of the range-finding study. No further information regarding the range-finding study was provided.

b. Definitive Study

Table 1. Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation Period:	3 days (pre-treatment period)	The chicks were 1 day old upon receipt, appeared in good health, and received no medication.
Conditions: (same as test or not)	Same as test	
Feeding:	Standard HRC chick diet in meal form (Batch 3) and Anglian Water (human potable), *ad libitum*	A detailed composition of the chick diet was provided.
Health: (any mortality observed)	No mortality was observed during the 3-day pre-treatment period.	

Parameter	Details	Remarks *Criteria*
Pen size and construction materials	Galvanized steel pens with a wire mesh floor measuring approximately 1.80 x 1.22 m.	Birds were housed in groups of ten (by treatment). The floor area was approximately 2196 cm^2 per bird, fulfilling the minimum recommended size of at least 600 cm^2 per chick.
Test duration	5 days with treated feed followed by 3 days with untreated feed	*Recommended test duration is 5 days with treated feed and at least 3 days observation with "clean" feed.*
<u>Test concentrations</u> nominal: measured:	0 (control), 650, 1300, 2600, and 5200 mg ai/kg diet <12 (<LOD, control), 680, 1310, 2540, and 5150 mg ai/kg diet	*A minimum of five concentrations are required for a definitive test.*
<u>Solvent/vehicle, if used</u> type: amount:	N/A	
Diet preparation and feeding	A premix of suitable strength was prepared by blending the required quantity of test compound with basal diet using a turbula mixer. The required concentrations were prepared by direct dilution of the premix. Blending of the inclusion levels for feeding was achieved by mixing in a double-cone blender for a minimum of 7 minutes. Diets were prepared 1 day prior to use.	*The control group should be tested with a diet containing the maximum amount of vehicle used in treated diets.*
Feed withholding period	None	
Stability and homogeneity of test material in the diet determined (Yes/No)	Yes	

Parameter	Details	Remarks *Criteria*
Number of birds per replicate/groups for negative control: for vehicle control: for treated:	10 birds/replicate N/A 10 birds/replicate	*The recommended number of birds per replicate is a minimum of ten.*
Number of replicates/group (if used) for negative control: for vehicle control: for treated:	2 replicates N/A 1 replicate/level	
Test conditions temperature: relative humidity(%): photoperiod:	22-24°C (mean daily min and max) 55% (mean daily) 12 hours light:12 hours dark	An infra-red lamp was suspended over each pen to provide an additional source of continuous heat. *14 hours light:10 hours dark or continuous lighting is required*
Reference chemical, if used	N/A	

2. Observations

Table 2. Observations

Parameters	Details	Remarks
Parameters measured (mortality/body weight/ mean feed consumption/ others)	Mortality Clinical signs of toxicity Body weight Food consumption Necropsy	

Parameters	Details	Remarks
Indicate the stability and homogeneity of test chemical in the diet	Stability of the test material in avian diet was assessed in the 163 and 5200 mg ai/kg diet levels after 6 days of storage under animal-room conditions. Recoveries were 99% and 103% of initial measured concentrations, respectively. Homogeneity was assessed by collecting samples from the top, middle, and bottom areas from treated feed prepared at 163 and 5200 mg ai/kg diet. Coefficients of variation were 1.5% and 1.4%, respectively.	
Indicate if the test material was regurgitated	No regurgitation was indicated.	
Treatments on which necropsies were performed	Any bird which died during the study was examined post-mortem. At study termination, necropsy was carried out on ten birds from the highest dose group in which there were survivors and on ten control birds.	*Gross necropsies should be performed on all birds that died and randomly selected survivors from all treatments including at least three control birds.*
Observation intervals	Birds were observed daily during the study and at frequent intervals during the treatment and post-treatment periods. Mortalities, bird health, and clinical signs were recorded at each observation. Group mean body weights were determined on Days -3, 0, 5, and 8. Group mean food consumption was determined over Days -3 to -1, 1 to 5 (daily), and 6 to 8.	
Were raw data included?	Yes	Group mean body weight and food consumption data are required.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortalities were observed during the 8-day study. The acute dietary LC_{50} >5200 mg ai/kg diet (nominal).

Table 3. Effect of Bitrex® (Denatonium Benzoate) on Mortality of Mallard Duck

Treatment **Mean-measured (nominal) concentration as mg ai/kg diet**		**No. of birds per treatment**	**Cumulative mortality**				
			day 1	**day 2**	**days 3-4**	**days 5-6**	**days 7-8**
Control		20	0	0	0	0	0
680 (650)		10	0	0	0	0	0
1310 (1300)		10	0	0	0	0	0
2540 (2600)		10	0	0	0	0	0
5150 (5200)		10	0	0	0	0	0
NOAEC		Not reported					
LC_{50}		>5200 mg ai/kg diet					
Reference chemical	mortality	N/A					
	LC_{50}	N/A					
	NOEC	N/A					

B. SUB-LETHAL TOXICITY ENDPOINTS

All birds remained in good health and no clinical signs of toxicity were observed. The excreta of all groups were normal in appearance throughout the study. Body weight increased in all groups and there was no clear evidence of a treatment-related effect. Food consumption was similar in all groups throughout the study. No abnormalities were detected in any bird examined *post-mortem.*

Table 4. Sublethal Effect of Bitrex® (Denatonium Benzoate) on Mallard Duck

Treatment Mean-measured (nominal) concentration as mg ai/kg diet		Observation					
		Body weight change, g			Food consumption, g/bird/day		
		Days -3 to 0	Days 0 to 5	Days 5 to 8	Days -3 to -1	Days 1 to 5	Days 6 to 8
Control		52	112	112	38	56	92
680 (650)		57	136	89	41	66	90
1310 (1300)		60	135	91	38	65	86
2540 (2600)		55	131	96	41	65	91
5150 (5200)		54	102	94	42	62	89
NOAEC		Not reported			Not reported		
EC_{50}		Not reported			Not reported		
Reference chemical	NOAEC	N/A					
	EC_{50}	N/A					

C. REPORTED STATISTICS

No statistical analyses were performed. Nominal concentrations were reported.

LC_{50} >5200 mg ai/kg bw 95% C.I.: N/A
Probit slope: N/A

D. VERIFICATION OF STATISTICAL RESULTS

Statistical analyses were not necessary because mortality was not observed. The NOAECs for mortality, body weight change, feed consumption, and clinical signs of toxicity were determined visually. All results are reported based on the mean-measured concentrations.

LC_{50} > 5150 mg ai/kg diet 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A

NOAEC = 5150 mg ai/kg diet
LOAEC > 5150 mg ai/kg diet

Endpoints Affected: None

E. STUDY DEFICIENCIES

There were no significant deviations from OCSPP Guideline 850.2200 affecting the scientific soundness or acceptability of this study.

F. REVIEWER COMMENTS

The reviewer's results agreed with the study authors'. However, the reviewer reported results in the Executive Summary and Conclusions sections in terms of mean-measured concentrations.

For method validation, the procedural recovery of Bitrex® from treated feed was determined prior to the definitive study at levels of 163 and 5200 mg ai/kg diet. Results indicated a mean procedural recovery of 97.3 ± 1.34% at the 163 mg ai/kg diet level and 93.9 ± 2.53% at the 5200 mg ai/kg diet level; results of samples analyzed were corrected for the "appropriate" mean procedural recovery value at analysis but were uncorrected for the purity of the test compound (100.2%).

Samples of treated feed were Soxhlet-extracted for 2 hours with acetonitrile, and an aliquot evaporated to dryness. The residue was re-dissolved in acetonitrile:0.02 M sodium chloride (80:20, v:v; mobile phase) using ultra-sonication. The final solution was filtered (0.45-µm) and the concentration of Bitrex® was determined using HPLC with UV (210 nm) detection.

There was reduced body weight change in the treatment groups compared to the control (mean of two replicates) from day 5-8 (14%-20%); however, there was no clear difference over the entire experimental period. Except for the highest test concentration, the body weight gain in the treatments was greater than the control from day 0 to 5. Mean body weight at the end of the experiment (day 8) was higher in treatment birds (except the highest treatment group) compared to the control. The two control replicate groups showed a fair amount of variability in weight change; thus, the mean body weight of the highest treatment group was reduced about 8% compared to the control mean (two replicates) but only about 3% compared to one of the control replicates. Overall, there was not a clear treatment related effect on body weight.

The definitive study was conducted January 23 (Day 0) to January 31, 1992.

G. CONCLUSIONS

This study is classified as **Acceptable** and satisfies the guideline requirement for an acute dietary toxicity study with mallard duck. There were no mortalities, clinical signs of toxicity, effects on body weight, or effects on feed consumption. In addition, no treatment-related gross pathological changes were observed.

LC_{50} >5150 mg ai/kg diet 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A

NOAEC = 5150 mg ai/kg diet
LOAEC > 5150 mg ai/kg diet

Endpoints Affected: None

III. REFERENCES

No references were cited.